Remarks:

Applicants have read and considered the Office Action dated February 25, 2003 in parent application Serial Number 09/508,891. Claims 1-3, 5-11 and 14 have been amended and claims 4, 12-13, 15-18 and 20 have been canceled. New claims 21 and 22 have been added. Claims 1-3, 5-11,14, 19, and 21-22 are pending.

Applicants have considered the Examiner's comments in item 2 of the Office Action regarding 37 CFR 1.3. Applicants note that while ''the finality of the previous Office Action has been withdrawn pursuant to 37 CFR 1.114'', nevertheless, a first and Final action following the filing of the RCE has been issued. Applicants thus filed a continuing application on August 25, 2003.

The original claims filed in the continuing application are amended so as to reflect the claims pending at the time the first and final rejection was issued on February 21, 2003.

Applicants submit two independent claims (claims 1 and 21) that relate to the identification of essential and non-essential genes under either selective or non-selective conditions. These claims are linked by the same unifying concept, which is submitted to be the identification of essential or non-essential genes and thus relate to the same invention. Applicants submit that the possibility of changing the growing conditions is an advantage and a testimony to the versatility and power of the present invention. For the purpose of clarity, Applicants have added new claim 22, which corresponds to claim 21 of the parent application 09/508,991 that was previously examined. Finally, claims 4, 12, 13, 15-18 and 20 have been canceled.

Additional support for saturation mutagenesis in the amended claims can be found at page 5, lines 1 to 4, and lines 23 and 24; at page 6, lines 16 to 19; at page 7, lines 4 to 6; at page 19, lines 23 to 27; at page 20, lines 25 to 29; as well as in Example 1 at page 26. Additional support for mutagenesis in general may be found for example at page 17, lines 8 to 31; and at

page 18, lines 1 to 6. Additional support for haploid genome may be found for example at page 1, lines 6 to 11; at page 4, lines 21 to 25; at page 9, lines 21 and 22; at page 19, lines 28 to 31; and at page 20, lines 1 to 18. Additional support for selective or specific conditions may be found for example at page 3, lines 27 to 30 and page 4, lines 1 and 2; at page 4, lines 14 to 17; at page 5, lines 27 to 29; at page 9, lines 13 to 15; and at page 18, lines 24 to 30. Additional support for vectors and bacteriophages may be found for example at page 15, lines 7 to 10; at page 19, lines 28 to 31; and at page 20, lines 1 to 18. Applicants assert that no new matter has been added.

Applicants note that the Action of February 25, 2003 stated that the response included language construed as complaints. Applicants apologize for any language construed as a complaint.

REJECTIONS UNDER 35 U.S.C. § 112 FIRST PARAGRAPH

Claims 4-12 and 21 were rejected under 35 U.S.C. § 112, first paragraph as ''containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention''. The Action refers to *Enzo Biochem Inc. v. Calgene, Inc.* (CAFC) 52 USPQ2d at 1135 bridging to 1136. The Action then refers to page 4, lines 5-8 of the specification:

> ''Accordingly, the present invention seeks to provide an essential gene test (EGT), an efficient and economical approach to define the function of thousands of sequences containing a complete open reading frame (ORF) or parts thereof, or known and/or unknown genes encoding hypothetical proteins or product''.

The Action states that the recited method steps of claim 4 do not result in the determination of any function of any sequence. The Examiner further contends that the claimed method allows one to determine at best whether one band of DNA is synthesized to a greater extent than a similar band from ''non-selected aliquots''. The Action concludes by stating that synthesis of a given band of DNA does not in and of itself define the function of the target

sequence that is the required end-point of the method of claims 4-12 and 21. Applicants respectfully traverse this rejection as follows.

Applicants have canceled claim 4, which refers to a function of a target sequence. Applicants have introduced new claim 21, which no longer refers to the identification of a function of a target region under selective conditions. Rather, new claim 21 refers to the identification of essential or non-essential target nucleotide sequences, which is well supported by the specification.

Moreover, Applicants respectfully submit that a function of the targeted DNA sequence can indeed be assessed by determining whether a targeted sequence is synthesized to a greater extent under selected conditions. A key element to assert a function of a targeted sequence according to the present invention is the kind of conditions used (selective or non-selective conditions). An example of a function according to the present invention is the essentiality of a sequence for survival or growth.

The present invention is based on the fact that transposon mutagenesis (TnM) within a non-essential nucleotide sequence (e.g. gene) of a haploid cell would not be expected to affect the growth rate of that cell, whereas TnM within an essential nucleotide sequence would result in a measurably diminished growth rate or fitness of the mutant compared to the wild type cell. As a consequence of this competitive growth disadvantage, the abundance of target sequence specific PCR products amplified from insertion mutations within such an essential sequence will be substantially reduced at certain time points. Thus, cells containing insertions in nucleotide sequences that are important for viability or fitness under specific growth conditions will not be represented in the outgrowth population. For example, if one wants to determine whether a specific bacterial sequence is essential or important for resistance to antimicrobial agents (i.e. if the function of the sequence is to confer resistance to antimicrobial agents), one would apply the method of the present invention in the presence and in the absence of an antibacterial agent and compare the amount and size of amplified targeted sequences in each condition. The presence of

two bands (one with a molecular weight that is higher than the expected control size (i.e. without an insertion) indicates that the targeted sequence is not essential under the selected conditions (e.g. in the presence of antimicrobial agents). On the other hand, the presence of only one band of expected size indicates that the function of the targeted sequence is to provide bacterial resistance to an antimicrobial agent.

Applicants submit that in accordance with the present invention, any growth condition can be selected in order to address a particular function of a sequence. For example, growth in the absence or presence of certain nutrients allows for determining whether a particular sequence is essential in specific metabolic pathways (e.g. in the absence of essential amino acids, in the absence of sugar...). Applicants emphasize that in one embodiment of the present invention, one type of growing condition is a so called ''non-selective'' condition. Under such condition one can determine, in accordance with the present invention, whether a targeted sequence is essential for survival or growth under normal proliferation circumstances. Thus, the growth conditions can be adapted in order to address whether a targeted sequence is essential in particular circumstances.

The conditions under which the methods of the present invention are performed are established in order to assess a particular function of a sequence. The Examiner is particularly referred to page 24 of the specification:

> ''In addition, the EGT assay can be utilized to dissect metabolic and genetic pathways by assessing mutagenized populations in different *in vitro* and *in vivo* conditions.''

Therefore, the methods of the present invention allow the identification of essential and non-essential genes under selective conditions and consequently make possible the identification of a function of a particular target sequence, under chosen selective conditions.

In any event, in order to expedite the prosecution of the instant case, Applicants have withdrawn without prejudice the terminology "function" from the claims. Applicants reserve the right to prosecute claims containing any canceled subject matter in further applications.

In view of the above and the foregoing, Applicants respectfully request that the rejection pertaining to the identification of a function of a target sequence be withdrawn.

The Action further states the following:

> ''Upon review of Example 1, it appears that the selective medium employed was the inclusion of Kanamycin in the culture media. However, the cells demonstrated resistance even after having undergone mutagenesis. Accordingly, the ''selective condition'' did not select for one group of cells over that of the other.''

Applicants respectfully assert that it appears from this extract of the comments that it may still not be clear to The Examiner what the test describes in Example 1, and what the purpose of Kanamycin is in this specific example. Applicants (Applicants have previously attempted to explain the same) assert that the purpose of including Kanamycin in this specific example is not to assess whether targeted sequences are essential for cell growth in the presence versus the absence of Kanamycin.

As is well known to a person of ordinary skill in the art, insertion of the miniTn5Km element into a genome disrupts the region into which it is inserted and confers resistance to Kanamycin (Km). Km is used as a general selection agent for cells that have incorporated the transposable element as opposed to cells that have not (i.e. general selection agent for cells of the library). It is used initially to select the library of mutagenized clones that have incorporated the transposable element; the number of Km resistant clones representing an estimation of the frequency of mutants obtained. Also, the use of Km throughout the assay reduces the chance of contamination with other cells (i.e. cells not initially in the library) and helps to keep the transposable element in the genome of cells that would otherwise tend to lose it. Since Applicants later discovered that the cells had become resistant to Kanamycin independently of

the presence of miniTn5Km, the experiments were repeated using another transposable element (miniTn5*tet*) that does not have a propensity to give false positive results (see page 27 of the disclosure).

The Action further states in item 5 that the disclosure fails to find adequate guidance for the analysis of any and all possible haploids, or the nucleic acids found therein, be they actual or theoretical polynucleotides. Therefore, it appears that under the standard used in the Action, burden of enablement of the claimed invention is shifted from the Applicant to the public. The Action cites for this matter *Genentech v. Novo Nordisk A/S* 42 USPQ2d 1001. Furthermore, the Examiner considers the instant disclosure as ''an invitation for others to develop the starting materials and reaction conditions that would enable the practicing of the claimed method for the full breadth of the claims' scope''.

Applicant agrees with the Examiner about the fact that ''the scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art'' [emphasis added]. Therefore, Applicant submits that in view of the common general knowledge in the art, a person of ordinary skill, could in fact easily adapt the method of the claimed invention to any known haploid genome.

The key element in order to adapt the methods of the present invention to any haploid genome is saturation mutagenesis, which is well described in the application. The Examiner is particularly referred to the definition of the term ''saturation mutagenesis'' beginning at page 21 of the disclosure:

> ''The term "saturation mutagenesis" as used herein with reference to a genome, refers to an insertion mutagenesis in substantially every gene thereof and/or every target region thereof. Based upon statistical analysis and well known methods, at least 90%, preferably, 95% and more preferably 100% of the genes and/or target regions will have been mutagenised. Briefly, to estimate the required conditions enabling the aiming of a complete population of mutagenised genes, the statistical analysis utilised is based on a number of criterions: 1) a completely random insertion of the insertion element (i.e. a mobile element); 2) an average size of 1 Kb for a typical gene in a prokaryote genome; 3) knowledge *a priori* of the genome size (Megabases). For example, a complete 1 X

> coverage of the *P. aeruginosa* 5.9 Mb genome would require a minimum of about 6000 clones after the mutagenesis experiment. Preferably, a minimum of 10 X coverage of the genome should be used by using 60,000 clones. When relating to DNA molecules present on a vector, saturation mutagenesis refers preferably to the insertion element being present at every nucleotide position thereof. It will be clear to a person of ordinary skill, to which the instant invention pertains, that the estimation of the conditions can be readily adapted to meet variations in the above-mentioned criterions or to meet particular needs should the criterions be different'' [Emphasis added].

The Examiner is further referred to page 17, lines 8 to 25 of the disclosure

> ''The mutagenesis of the DNA or of the cells is carried out in accordance with well-known methods (Sambrook et al., 1989, supra), such that the total DNA population or cell population has statistically at least one insertion mutation in each and every gene of the genome. Essentially, the one tube collection of mutants obtained by mutagenesis covers the complete genome. A typical mutagenesis experiment can yield mutants at frequencies varying from 10,000 clones to more than 1,000,000 clones. Such mutants can be recovered in a single tube. *This mutagenesis scheme is based on the premise that the genome size is known, that mutagenesis is a random event and that a typical gene has an average size of 1 kilobase. For example and on a statistical basis, the 5.9 Mb Pseudomonas aeruginosa genome would require a minimum of 5,900 mutants to cover the genome at least once. This is herein defined as a 1 X genome coverage.* Thus, a collection of 17,500 mutants (3 X), 29,500 mutants (5 X) or 59,000 mutants (10X) could be utilized for screening in a typical EGT assay for this particular microorganism. Of course, and as shown in Example 2, the person of ordinary skill could also screen more than 10X. The person of ordinary skill will be able to adapt the present teachings to suit particular needs and adapt the instant invention to chosen genomes and specifics thereof'' [Emphasis added].

Thus, the only step of the methods that needs to be adapted in order to apply the methods of the present invention to any haploid organism is the saturation mutagenesis step, provided that the genome size and the nucleotide sequence or portion thereof of the target region of interest is known (see page 19 lines 27 of the disclosure). The present disclosure clearly explains and establishes the particular parameters that must be varied in order to adapt the present invention to any haploid genome. This step is well defined in the application and has been known and widely applied for years by scientists around the world. Limiting the claims to a particular haploid genome would unfairly restrict their scope since it can easily be adapted without undue experimentation. Therefore, the limitation would unjustly allow a large number of people to take advantage of the present invention.

In view of the above and foregoing, Applicants respectfully request that the rejection regarding enablement for any haploid genome be withdrawn.

The Action further indicates in item 6 that "with the claimed method ultimately having utility in the properties of the expressed proteins, one needs to have some capacity to predict the outcome of such experiments". The Action further states that "the claimed invention relates directly to matters of physiology and chemistry, which are inherently unpredictable and as such, require greater levels of enablement". In addition, the Examiner refers to US Patent Publication 20030033625, to state that ''it is difficult to determine gene function after a knockout event since one could not discern whether a given phenotype is associated with the inactivation of a gene, or the transcription of nearby genes''. Applicants traverse this rejection as follows.

Applicants respectfully submit that the claimed invention does not need to have some capacity to predict the outcome of such experiments. In fact, one of the main objects of the present invention is to rapidly and efficiently identify whether targeted sequences are essential or not under specific conditions without a need for prediction. Therefore one does not need to predict anything about the function of a sequence in order to practice the methods of the present invention. As disclosed in the application, one only needs to know the size of the genome and a limited portion of the nucleotide sequence of the target sequences of interest.

Additionally, it should be noted that the methods of the present invention do not necessarily apply to nucleotide sequences encoding proteins or open reading frames. The Examiner is particularly referred to page 19 of the disclosure, starting at line 8:

> **''It will also be understood that the instant invention is not limited to the identification of essential ORFs. A person of ordinary skill will understand that insertions into 5' and 3' non-coding sequences could also be shown to be detrimental or fatal to the survival of a cell harboring such an insertion**. Thus, the present invention also covers the identification of DNA targets which are essential under selective or non-selective conditions''.[Emphasis added]

Thus, according to the present invention, the essentiality of any targeted sequence (encoding a protein or not) can be assessed by using the method of the present invention. Therefore, one could assess if an untranslated region of a gene is essential or not for the growth of cells.

By specifying the growth conditions of mutant cells of the library, one can determine whether target sequences are essential or dispensable under such specific conditions. For example, the essentiality of a sequence for the metabolism of specific nutrients (e.g. specific amino acids) can be assessed by growing the cells in the absence of such nutrients. One could also assess whether a sequence is essential for bacterial resistance by growing the mutagenized bacteria in the presence versus the absence of antimicrobial agents.

Thus, while only having a hypothesis concerning a function of a sequence, the sequence can be tested in accordance with the assays of the present invention. A so-called ''shot-gun'' approach, in which for example, different growth conditions can be used and the method of the present invention can be tested under such different conditions to identify essential sequences under specific growth conditions, can also be used. The present invention provides tools to enable one skilled in the art, not only to determine whether a targeted sequence is essential (or not) for cell survival or growth in normal conditions, but also to determine a certain function by assessing the essentiality of the sequence in selected conditions (e.g. in the presence of antimicrobial agents, in the absence or presence of certain nutrients, or chemicals etc.). According to the present invention, the growth conditions are set in order to assess the essentiality of the target sequences and are well known to those of ordinary skill in the art.

In any event, Applicants stress and reiterate that the determination of a function of the inserted sequence is no longer claimed, even though the present invention enables a determination of some functional aspects thereof.

As for knock out being unpredictive of the function of a protein, Applicants respectfully submit the following. Gene knock out is one of the most powerful tools of contemporary molecular biology. It is used by hundreds of laboratories around the world and several large scale projects are funded in order to create large libraries of mutant species where each and every gene would be mutated. Knocking out is one of the most powerful techniques enabling one to obtain knowledge about the functions of a targeted sequence. In fact, most knock out experiments having selection sequences were found to be successful. This method is still frequently used to generate knock out mice. Moreover, the effects of several commonly used sequences employed for selection of positive cells are now well documented. Therefore commonly used sequences that are less susceptible to produce side effects and strategies to reduce these effects are also well known in the art and could readily be adapted to the methods of the present invention.

Additionally, it is respectfully submitted that Applicants should not be deprived of having a patent granted because of a contention that the method of the present invention has some limitations in particular conditions. One of the purposes and basic principles of the patent system is to enable improvement of the technology and to promote the useful arts and sciences. The patent system is meant to encourage the publication of new scientific work, so that science and technology can develop because of it. The present invention achieves these purposes. Therefore, others can improve upon the invention disclosed in order to promote advances in the technology.

In view of the above and the foregoing, Applicants respectfully request that the rejection relating to the unpredictability of the claimed methods be withdrawn.

The Action states that claim 9 relating to mutagenized DNA molecules cloned into a bacteriophage, lacks enablement. The Action states that ''in such case the sequence is not part of the genome of the organism, but is part of an extra-chromosomal element'' and that the specification does not set forth a reproducible method whereby the function of the sequence could be readily determined simply by determining the levels of nucleic acid synthesis from one population over that of the other. Applicants traverse this rejection as follows.

Applicants respectfully refer to page 19 of the disclosure, beginning at line 28:

> "Although in a preferred embodiment, the present invention is adapted for use with a whole genome, a DNA molecule inserted into a vector can also be used in accordance with the present invention. In such an embodiment, the vector should permit an expression of the DNA molecule in order to permit an assessment of the essentiality of the gene product. In such a scheme, it will be understood that only **dominant insertional mutation can provoke the lethality since, presumably, a copy of a wild type or homologous copy of the gene which is present on the vector, is present in the host cell.** Consequently, it will be clear to the person of ordinary skill that although the present invention is not limited to haploid genomes, the method of the present invention is favorably used in a context of a haploid organism, more preferably a haploid microorganism and especially in Gram positive and Gram negative bacteria. Organisms in which conversion to homozygocity is efficient and/or complete are also covered by the scope of the present invention. In a preferred embodiment therefore, prokaryotic genomes and lower eukaryotic genomes such as the haploid genomes of parasites and protista are used. Non-limiting examples of such lower eukaryotic genomes include that of tachyzoite form of *Toxoplasma gondii*, of *Plasmodia*, *Schistosoma* and *Leishmania* species, as well as those of fungi such as that of *Candida*, *Aspergillus*, *Neospora* and other disease causing (in plants, in animals and in humans) relevant fungi are especially preferred genomes. In addition, all disease causing agents such as Influenzae, HIV, Herpes and other viruses may also be used in the context of the present invention".

Therefore, according to the citation above, as long as the mutation is a dominant insertional mutation, libraries cloned into vectors (including bacteriophages), may be used according to the present invention. Libraries cloned into vectors could be used, for example, when studying the essentiality of viral genomes. Since viral sequences are not normally present in normal cells, the introduction of targeted sequences cloned into vectors makes the cell haploid for such sequences. Thus, according to the present invention, sequences could be insertionally mutated, cloned into vectors, transformed into cells and maintained as extra-chromosomal elements. Then, the essentiality of viral sequences could be assessed by comparing cells comprising the mutated sequences with cells comprising the wild type sequences. As stated above, the method of the present invention can be used as long as no other copy of the target sequence exists in the host cell, thereby masking the insertional mutation of a first copy of the target sequence.

Additionally, Applicants submit that cloning methods into filamentous bacteriophages bearing regulatory sequences for expression of target sequences are very well known in the art.

Such methods may be found for example in chapter four of ''Sambrook et al., 1992, Molecular Cloning, a laboratory Manual- 2nd Edition, CSH Laboratories''.

In view of the above and foregoing, it is respectfully requested that the rejection of claim 9 under 35 U.S.C. § 112, first paragraph be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 101

Claims 4-12 and claim 21 were rejected under 35 U.S.C. § 101, alleging that the claimed invention is not supported by either a credible asserted utility or a well established utility. The Action first states that the specification asserts that the claimed method can result in the identification of therapeutic agents as well as the identification of the function of genes of the organism. The Action further states that in order for the claimed method to meet the utility requirements, either the method itself must have a specific, well-established or credible utility, or the method results in a product that in turn has such utility. In the Action it is contended that a review of the specification fails to find where any function has been found of a target sequence that would meet the utility requirement and the specification fails to disclose where any such functional analysis of a target sequence has resulted in the identification of any product, therapeutic or otherwise, that satisfies the utility requirement. The rejection concludes by stating that since the claimed invention is not supported by a credible asserted utility, that one skilled in the art would clearly not know how to use the claimed invention.

Applicants respectfully disagree with the Examiner's conclusion that the present invention is not supported by a credible asserted utility or well-established utility. Moreover, Applicants respectfully submit that the alleged lack of utility rejection has been rendered moot by the amendments to the claims, which now recite methods for ''identifying essential and non essential target nucleotide sequences''. In any event, Applicants respectfully traverse the utility rejection as follows.

Applicants submit that the present invention provides methods for the identification of essential genes in haploid cells, in particular in the context of microbial genomics. The essential gene test (EGT) of the present invention is a transposon-based technique that can rapidly identify a nucleotide sequence from a database as being essential or dispensable. Moreover, the present invention also enables one to determine the essentiality of nucleotide sequences in particular growth conditions (e.g., particular temperature, drug, or chemicals) and therefore assesses a particular function of a sequence under such selected conditions. Thus, the present invention could be used, for example, to identify genes that are essential for drug resistance. By identifying the existence of essential sequences required for the growth and survival of a pathogenic organism (e.g. bacteria, viruses...), the present invention provides new tools for the identification of essential genes. Consequently, the present invention finds credible utility for example, in discovering antibacterial targets and vaccine candidates.

Moreover, the value of identifying whether a target sequence is essential or not in a haploid pathogenic organism is well established in the scientific community. In fact, several papers describing transposon-based approaches for the identification of essential genes from various laboratories around the world have been published in well recognized peered reviewed journals as prestigious as *Nature biotechnology*[1]. The Examiner is particularly referred to a review article by two of the three inventors of the present invention, on the discovery of essential and infection-related genes[2] that addresses thoroughly the utility of the EGT test and related transposon-based methods. Obviously, the clear identification of a repertoire of essential genes by a simple method such as EGT will be utilized in the next phase of drug development and control of infectious diseases. These approaches form the basis for the identification of microbial

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[1] Judson N, Mekalanos JJ: TnAraOut, a transposon-based approach to identify and characterize essential bacterial genes. *Nat Biotech* 2000, 18:740-745.

[2] LeHoux DE, Sanchagrin F and Levesque RC. Discovering essential and infection-related genes. *Curr Opin Microbiol* 2001, 4:515-519.

targets in the development of novel antimicrobials and vaccines by the biotechnology and pharmaceutical industry and are therefore extremely useful to the community.

Hence the utility of the EGT test in the identification of essential genes and therefore potential drug targets is not simply a wish list of unfounded applications, but a utility that has been reasonably confirmed by experimental results presented in the application in Example 1, as well as in other later published papers.

Further, Applicants submit that any discovery constitutes a useful new point of departure for additional research:

> ''As long as one specific substantial and credible use is disclosed and the statutory requirements are met, the USPTO is not authorized to withhold the a patent until a another, or better use is discovered. The researchers may discover higher or better or more practical uses, but they are advantaged by the starting point that the original disclosure provides.'' (Federal Register vol. 66, No. 4 p. 1094, in response to comment 7).

Finally, as stated in the Federal Register, the disclosure of a single utility is sufficient to satisfy the utility requirement:

> ''The patentee is required to disclose only one utility, that is, teach others how to use the invention in at least one way. The patentee is not required to disclose all possible uses, but promoting the discovery of other uses is one of the benefits of the patent system. When patent for genes are treated the same as for other chemicals, progress is promoted because the original inventor has the possibility to recoup research cost, because others are motivated to invent around the original patent, and because a new chemical is made as a basis for future research''. (Federal Register vol. 66, No. 4 p. 1094, in response to comment 5). [Emphasis added]

In view of the above, Applicants submit that the present claims more than satisfy the utility requirement and respectfully request that the rejection under 35 U.S.C. § 101 be withdrawn.

The rejections of the original claims are believed to now have been overcome. From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such an action is earnestly solicited. If the Examiner feels that a telephone interview may be helpful in this matter, please contact Applicants' representative at 612.336.4728.

Respectfully submitted,

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